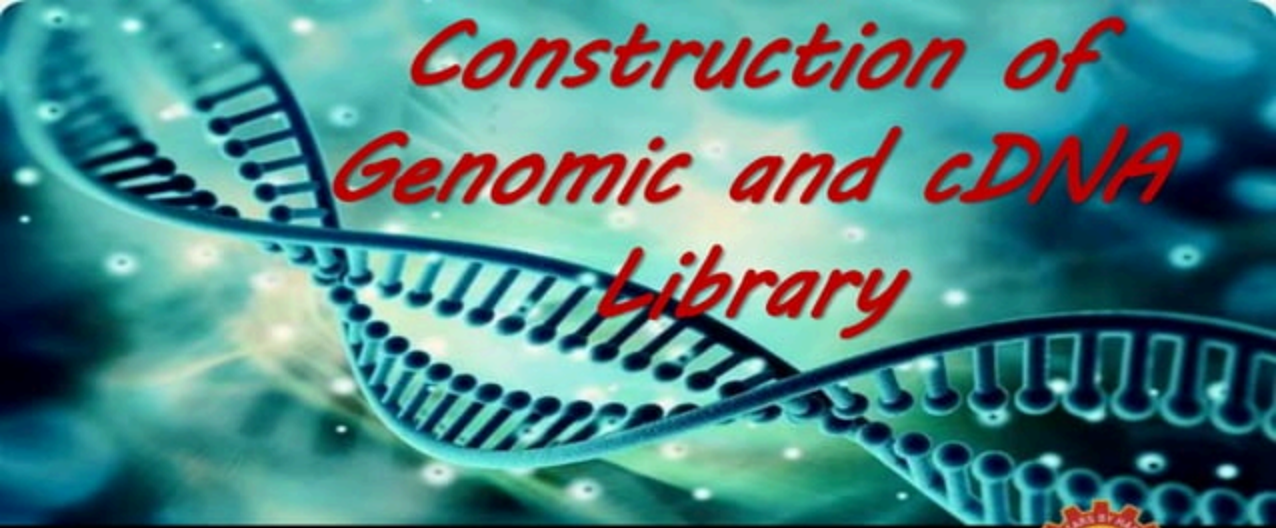

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INTRODUCTION

- The use of genetic information is a powerful tool that today is becoming more readily available to scientists.
- In order to use this powerful tool it necessary to know how to navigate throughout the entire genome. The human genome is about 3 x 10E9 bp.
- In humans this project is known as Human Genome Project

GENOMIC AND CDNA LIBRRARY

What is Genomic library?

A genomic library is a collection of the total genomic DNA from a single organism. (exons and introns).

What is cDNA library?

A cDNA library is a combination of cloned cDNA (complementary DNA) fragments inserted into a collection of host cells, which constitute some portion of the transcriptome of the organism and are stored as a "library"

It contains only expressed genomic information (only exons)

Genomic library

- **A genomic library** is a collection of the total genomic DNA from a single organism. The DNA is stored in a population of identical vectors, each containing a different insert of DNA. In order to construct a genomic library, the organism's DNA is extracted from cells and then digested with a restriction enzyme to cut the DNA into fragments of a specific size. The fragments are then inserted into the vector using DNA ligase. Next, the vector DNA can be taken up by a host organism - commonly a population of Escherichia coli or yeast - with each cell containing only one vector molecule. Using a host cell to carry the vector allows for easy amplification and retrieval of specific clones from the library for analysis.

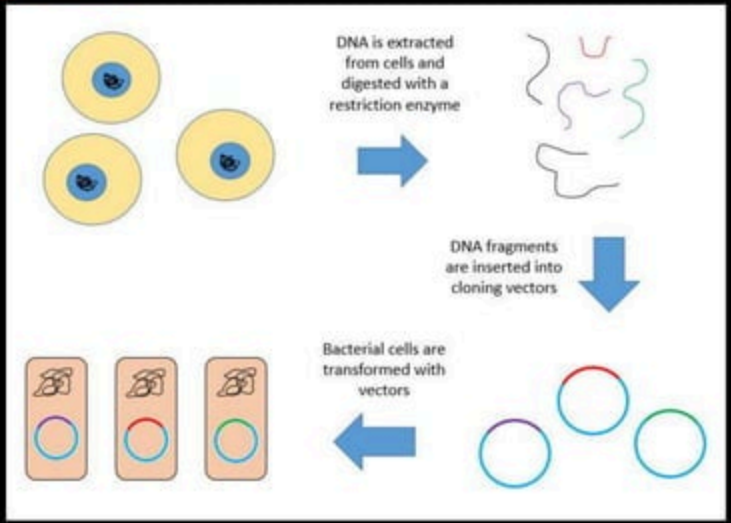
Genomic library

- There are several kinds of vectors available with various insert capacities. Generally, libraries made from organisms with larger genomes require vectors featuring larger inserts, thereby fewer vector molecules are needed to make the library.
- Researchers can choose a vector also considering the ideal insert size to find the desired number of clones necessary for full genome coverage.
- Genomic libraries are commonly used for sequencing applications. They have played an important role in the whole genome sequencing of several organisms, including the human genome and several model organisms.

Construction of Genomic library

- Construction of a genomic library involves creating many recombinant DNA molecules. An organism's genomic DNA is extracted and then digested with a restriction enzyme.
- For organisms with very small genomes (~10 kb), the digested fragments can be separated by gel electrophoresis. The separated fragments can then be excised and cloned into the vector separately. However, when a large genome is digested with a restriction enzyme, there are far too many fragments to excise individually.
- The entire set of fragments must be cloned together with the vector, and separation of clones can occur after. In either case, the fragments are ligated into a vector that has been digested with the same restriction enzyme. The vector containing the inserted fragments of genomic DNA can then be introduced into a host organism.

Construction of Genomic Library



This is a diagram of the above outlined steps.

Construction of Genomic Library

1· Preparing DNA:

- The key to generating a high-quality library usually lies in the preparation of the insert DNA· The first step is the isolation of genomic DNA· The procedures vary widely according to the organism under study· Care should be taken to avoid physical damage to the DNA·
- If the intention is to prepare a nuclear genomic library, then the DNA in the nucleus is isolated, ignoring whatever DNA is present in the mitochondria or chloroplasts· If the aim is to make an organelle genomic library, then it would be wise to purify the organelles away from the nuclei first and then prepare DNA from them·

Construction of Genomic Library

2· Fragmentation of DNA:

- ✓ The DNA is then fragmented to a suitable size for ligation into the vector· This could be done by complete digestion with a restriction endonuclease· But this has a demerit· Digestion by the use of restriction endonuclease produces DNA fragments which are not intact·
- ✓ To solve this problem we use partial digestion with a frequently cutting enzyme (such as Sau3A, with a four-base-pair recognition site) to generate a random collection of fragments with a suitable size distribution·
- ✓ Once prepared, the fragments that will form the inserts are often treated with phosphate, to remove terminal phosphate groups· This ensures that separate rate pieces of insert DNA cannot be ligated together before they are ligated into the vector· Ligation of separate fragments is undesirable, as it would generate clones containing non-contiguous DNA, and we would have no way of knowing where the joints lay·

Construction of Genomic Library

3. Vector Preparation:

This will depend on the kind of vector used. The vector needs to be digested with an enzyme appropriate to the insert material we are trying to clone.

4. Ligation and Introduction into the Host:

Vector and insert are mixed, ligated, packaged and introduced into the host by transformation, infection or' some other technique.

Construction of Genomic Library

5· Amplification:

- This is not always required· Libraries using phage cloning vectors are often kept as a stock of packaged phage· Samples of this can then be plated out on an appropriate host when needed· Libraries constructed in plasmid vectors are kept as collections of plasmid-containing cells, or as naked DNA that can be transformed into host cells when needed·
- With storage, naked DNA may be degraded· Larger molecules are more likely to be degraded than smaller ones, so larger re-combinants will be selectively lost, and the average insert size will fall·

Types of Vectors used in Genomic Library

Genome size varies among different organisms and the cloning vector must be selected accordingly. For a large genome, a vector with a large capacity should be chosen so that a relatively small number of clones are sufficient for coverage of the entire genome.

However, it is often more difficult to characterize an insert contained in a higher capacity vector.

Types of Vectors used in Genomic Library

Vector type	Insert size (thousands of bases)
Plasmids	up to 10
Phage lambda (λ)	up to 25
Cosmids	up to 45
Bacteriophage P1	70 to 100
P1 artificial chromosomes (PACs)	130 to 150
Bacterial artificial chromosomes (BACs)	120 to 300
Yeast artificial chromosomes (YACs)	250 to 2000

STORAGE OF GENOMIC LIBRARY

- Once a genomic library has been made it forms a useful resource for subsequent experiments as well as for the initial purpose for which it was produced. Therefore, it is necessary to store it safely for future use. A random library will consist of a test tube containing a suspension of bacteriophage particle (for a phage vector).
- The libraries are stored at – 80°C. Bacterial cells in a plasmid library are protected from the adverse effects of freezing by glycerol, while phage libraries are cryoprotected by dimethyl sulfoxide (DMSO).

Applications of Genomic Library

Genomic library has following applications:

1. It helps in the determination of the complete genome sequence of a given organism.
2. It serves as a source of genomic sequence for generation of transgenic animals through genetic engineering.
3. It helps in the study of the function of regulatory sequences in vitro.
4. It helps in the study of genetic mutations in cancer tissues.
5. Genomic library helps in identification of the novel pharmaceutical important genes.
6. It helps us in understanding the complexity of genomes.

cDNA Library

A **cDNA library** is a combination of cloned cDNA (complementary DNA) fragments inserted into a collection of host cells, which constitute some portion of the transcriptome of the organism and are stored as a "library". cDNA is produced from fully transcribed mRNA found in the nucleus and therefore contains only the expressed genes of an organism.

Similarly, tissue-specific cDNA libraries can be produced. In eukaryotic cells the mature mRNA is already spliced, hence the cDNA produced lacks introns and can be readily expressed in a bacterial cell.

While information in cDNA libraries is a powerful and useful tool since gene products are easily identified, the libraries lack information about enhancers, introns, and other regulatory elements found in a genomic DNA library.

Construction of cDNA Library

cDNA is created from a mature mRNA from a eukaryotic cell with the use of reverse transcriptase. In eukaryotes, a poly-(A) tail (consisting of a long sequence of adenine nucleotides) distinguishes mRNA from tRNA and rRNA and can therefore be used as a primer site for reverse transcription. This has the problem that not all transcripts, such as those for the histone, encode a poly-A tail.

1. mRNA extraction:

Firstly, the mRNA is obtained and purified from the rest of the RNAs. Several methods exist for purifying RNA such as trizol extraction and column purification. Column purification is done by using oligomeric dT nucleotide coated resins where only the mRNA having the poly-A tail will bind. The rest of the RNAs are eluted out. The mRNA is eluted by using eluting buffer and some heat to separate the mRNA strands from oligo-dT.

Construction of cDNA Library

2. cDNA construction:

Once mRNA is purified, oligo-dT (a short sequence of deoxy-thymidine nucleotides) is tagged as a complementary primer which binds to the poly-A tail providing a free 3'-OH end that can be extended by reverse transcriptase to create the complementary DNA strand.

Now, the mRNA is removed by using a RNAse enzyme leaving a single stranded cDNA (sscDNA). This sscDNA is converted into a double stranded DNA with the help of DNA polymerase. However, for DNA polymerase to synthesize a complementary strand a free 3'-OH end is needed.

This is provided by the sscDNA itself by generating a hairpin loop at the 3' end by coiling on itself. The polymerase extends the 3'-OH end and later the loop at 3' end is opened by the scissoring action of S, nuclease. Restriction endonucleases and DNA ligase are then used to clone the sequences into bacterial plasmids.

The cloned bacteria are then selected, commonly through the use of antibiotic selection. Once selected, stocks of the bacteria are created which can later be grown and sequenced to compile the cDNA library.

Types of vectors used in cDNA Library

Both the bacterial and bacteriophage DNA are used as vectors in the construction of cDNA library.

Vectors	Insert size
λ-phage	Up to 20-30kb (for replacement vectors) and 10-15kb (for insertion vectors)
Bacterial plasmids	Up to 10-15kb

Advantages of cDNA Library

A cDNA library has two additional advantages·

- ✓First, it is enriched with fragments from actively transcribed genes·
- ✓Second, introns do not interrupt the cloned sequences; introns would pose a problem when the goal is to produce a eukaryotic protein in bacteria, because most bacteria have no means of removing the introns·

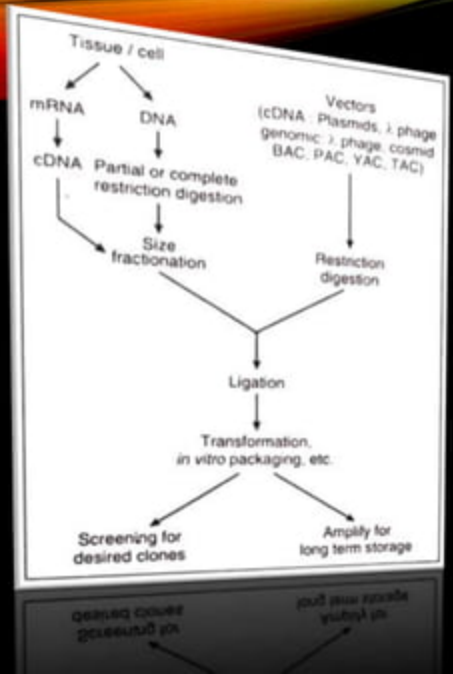
Disadvantages of cDNA Library

The disadvantage of a cDNA library is that it contains only sequences that are present in mature mRNA. Introns and any other sequences that are altered after transcription are not present; sequences, such as promoters and enhancers, that are not transcribed into RNA also are not present in a cDNA library.

Applications of cDNA library

Following are the applications of cDNA libraries:

1. Discovery of novel genes.
2. Cloning of full-length cDNA molecules for in vitro study of gene function.
3. Study of the repertoire of mRNAs expressed in different cells or tissues.
4. Study of alternative splicing in different cells or tissues.



Flow chart showing the construction of Genomic library and cDNA library


Thank
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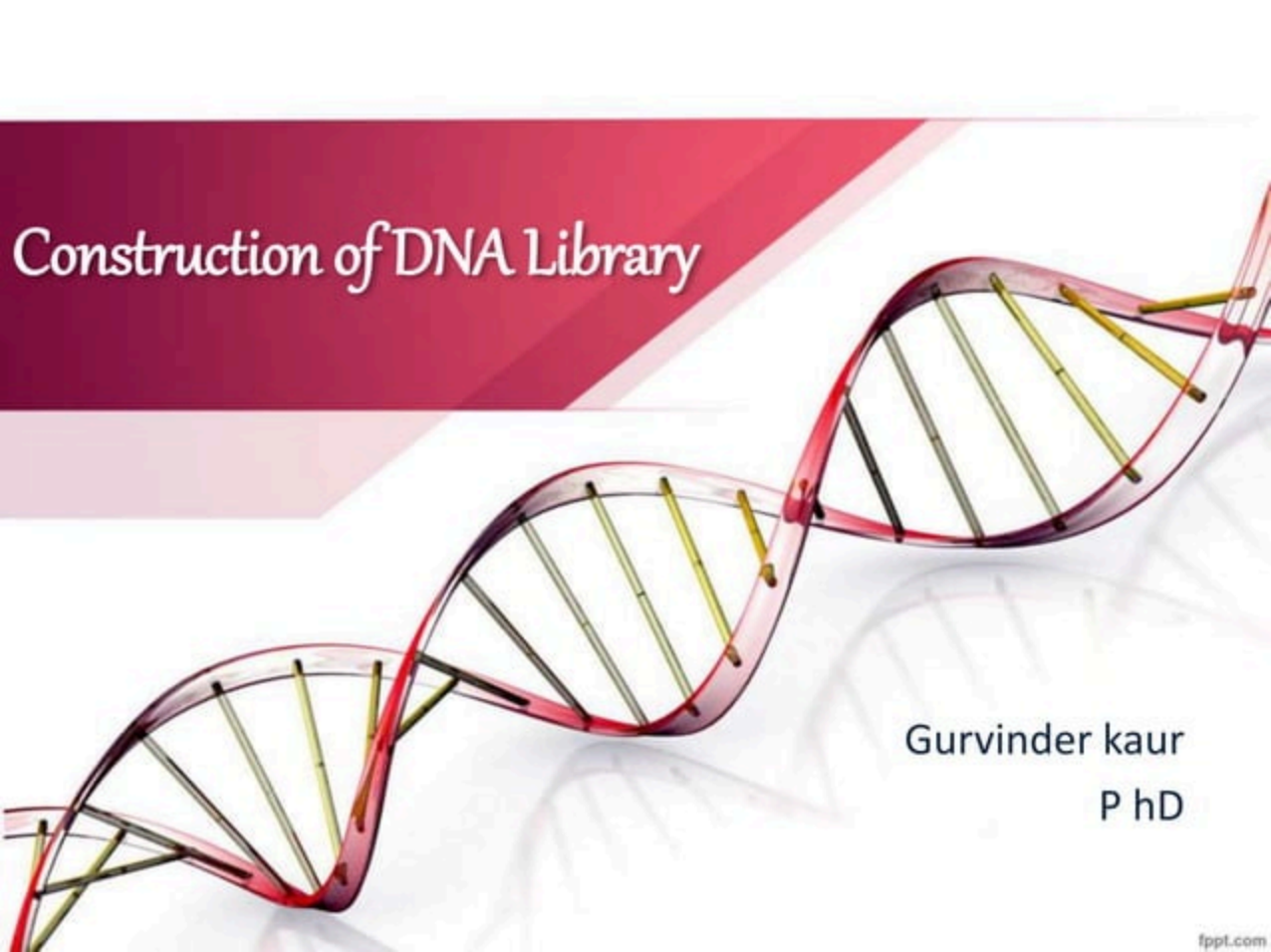
Construction of DNA Library



Gurvinder kaur

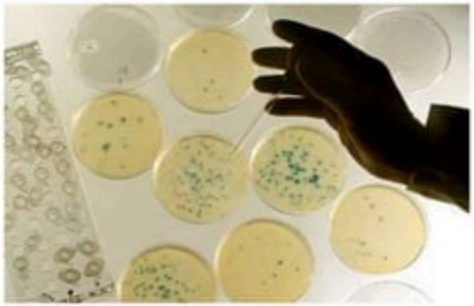
P hD

What is DNA library?

- Collection of **DNA** fragments that have been cloned into vectors so that researchers can identify and isolate the **DNA** fragments that interest them for further study.

- In molecular biology, a **library** is a collection of DNA fragments that is stored and propagated in a population of micro-organisms through the process of molecular cloning.

What are libraries for?

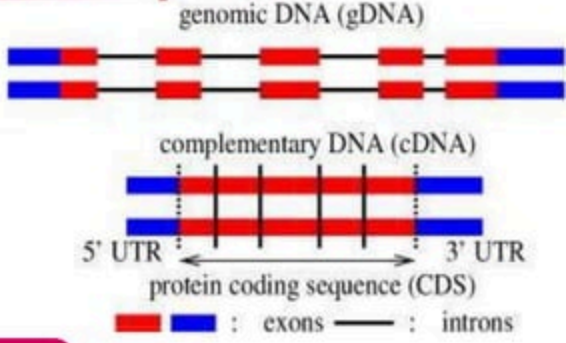


- Storage/ store many copies of a gene
- Host most commonly *e.coli* are use for storing whole genomic data in the form of bacterial colonies
- Collection of all the clonned vectors
- Eg genomic library in a petri dish.

Why we require libraries?

- In order to study a gene, a researcher needs to isolate it from all the other genes in an organism's **DNA**
- To make the research easier
- Once, we have a library, we can locate it by various screening methods and can use it for various research purposes
- **Genomic libraries** are commonly used for sequencing **applications**.
- Ease of purification, storage and analysis.

DNA library



- DNA library
  - Genomic library
  - cDNA library

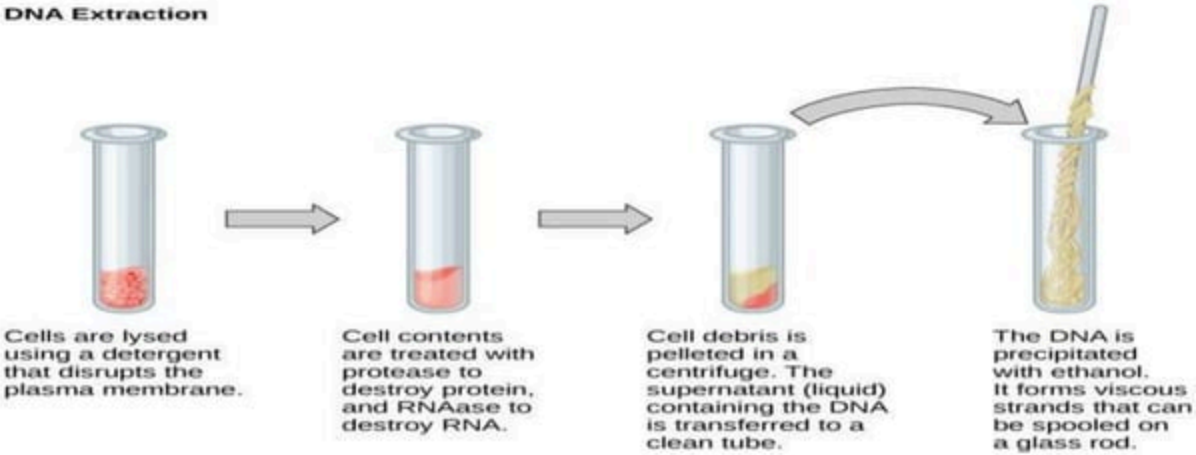
Genomic Library

- Contains DNA fragments representing entire genome of an organism
- Created using molecular cloning
- vectors are engineered to carry the DNA fragments.
- vector genome containing the foreign insert is replicated, producing clones of the original genome
- This collection of clones, in theory contains all sequences found in the original source, including the sequence of interest
- Genomic libraries can be constructed using various hosts like plasmids, bacteriophage lambda and many more.

Steps of genomic library construction

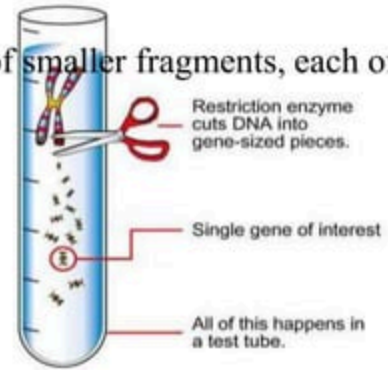
1. Isolation of DNA from cells
2. Digestion into small fragments
3. Introduction into suitable vectors
4. Insertion into bacteria
5. Production/identification of clones
6. Collection of Genomic DNA library

1. Isolation of DNA

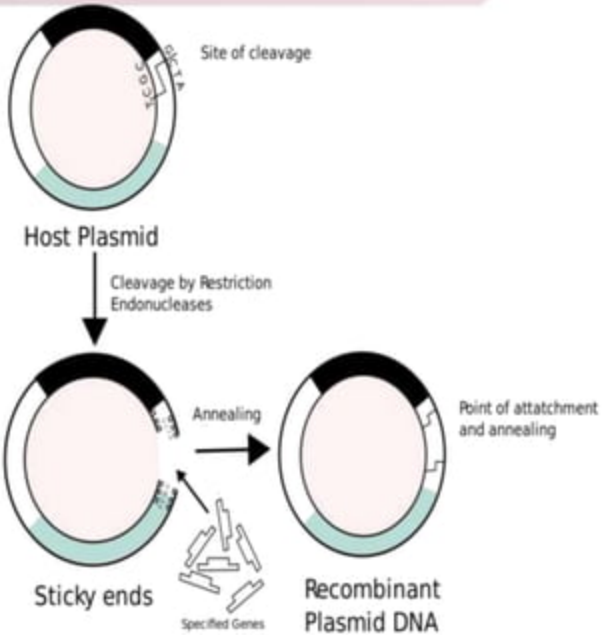


2.Digestion into small fragments

- Purified DNA consist of extremely long strands
- To begin, the strands must first be cut into manageable sizes
- Physical shearing: pipetting,, mixing
- Restriction enzyme digestion- partial digestion is preferred to get a greater lengths of DNA fragments.
- The restriction enzyme cut the DNA into 1000s of smaller fragments, each of which may contain one or more gene.
- Selection of restriction enzymes is very critical

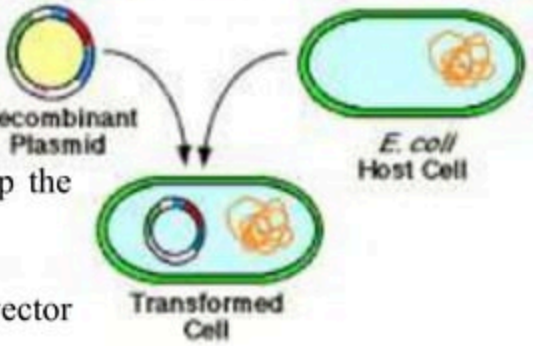


3.Introduction into suitable vectors



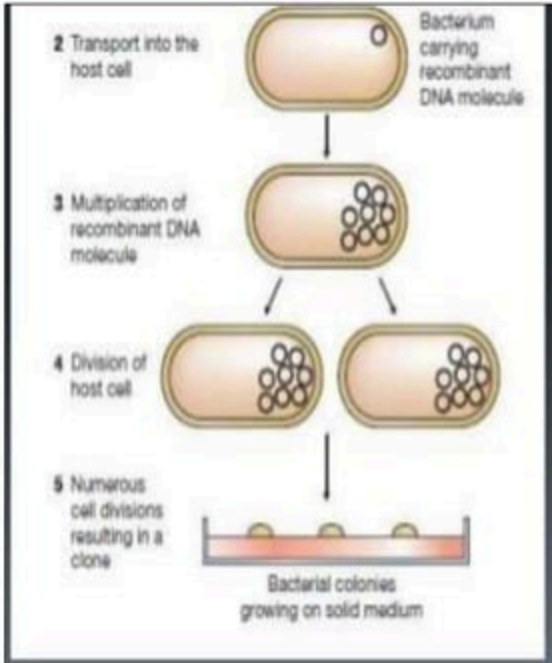
- Each fragment is different and have a unique DNA sequence
- inserted into suitable vectors including plasmids and bacteriophage vectors
- Vectors are digested with the same Restriction enzymes and sealed to human DNA using DNA ligase enzyme.
- The resulting molecules are recombinant.

4.Insertion into host



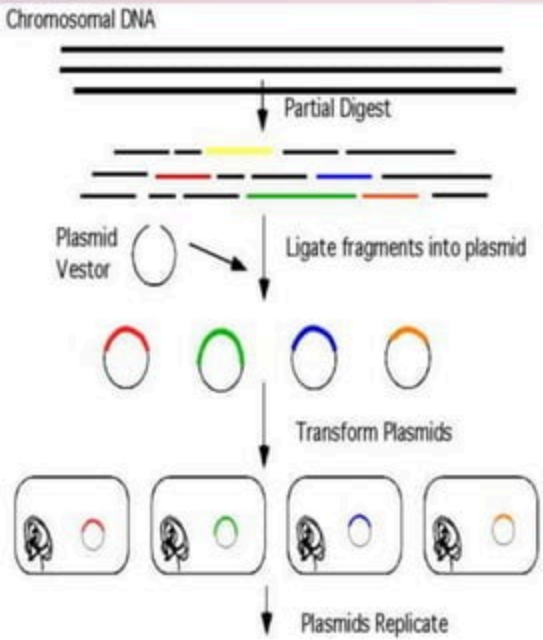
- Inserted into host bacteria (E.coli)
- Bacterial cells are made competent to take up the DNA.
- They replicate their genome along with the vector genome contained with them.
- Produce clones of the original genome
- This collection of clones which contains all the sequences, including the sequence of interest forms the genomic library.

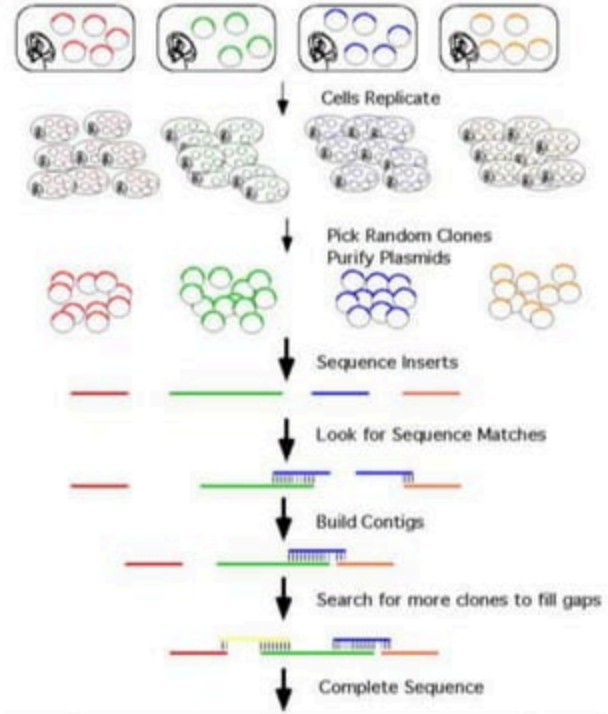
Multiplication and production of clones



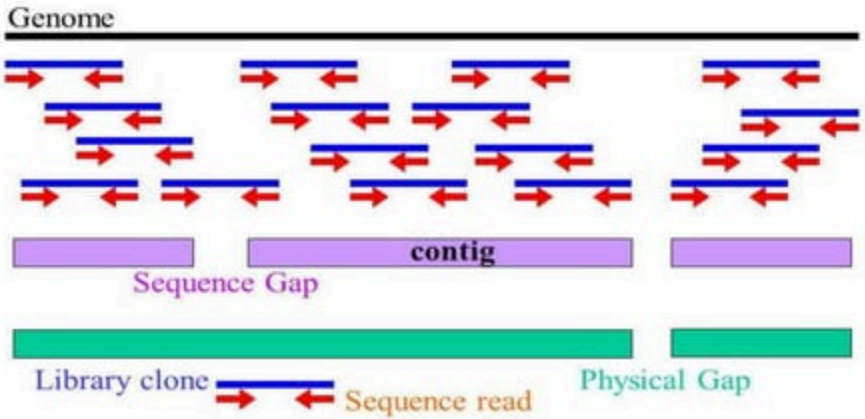
After a large number of cell divisions, a colony, or clone, of identical host cells is produced. Each cell in the clone contains one or more copies of the recombinant DNA molecule; the gene carried by the recombinant molecule is now said to be cloned.

Genomic DNA Library-overview





Contig



Genomics: 24

cDNA Library

✓ Libraries that represent the mrna in a particular cell or tissue are termed cdna libraries.

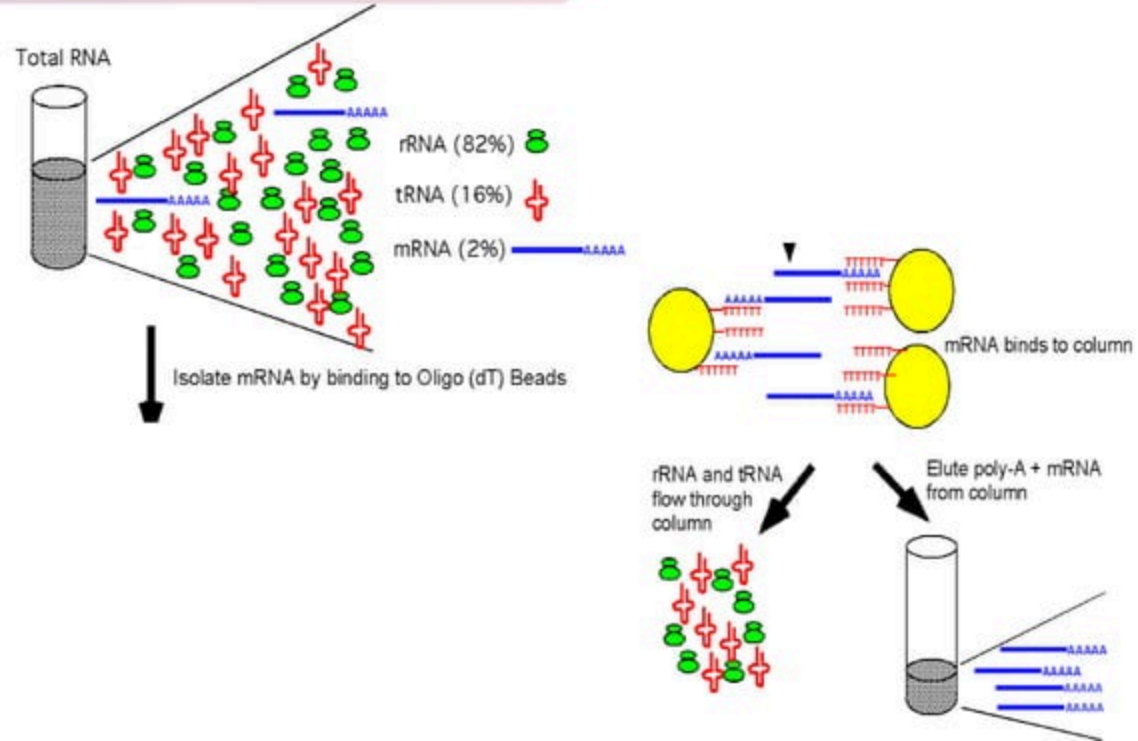
✓DNA copies derived from the mrna molecules

✓This process is accomplished using enzyme reverse transcriptase, which is isolated from RNA- containg retroviruses.

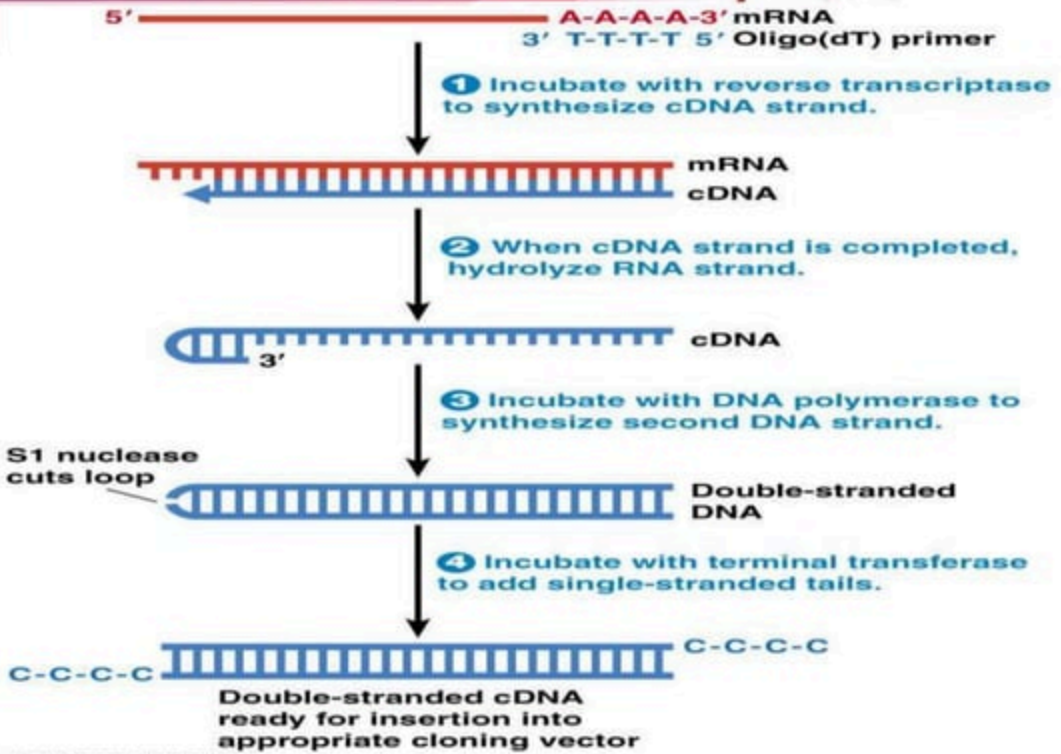
✓Cdna is synthesized in two steps from mrna molecule.

✓The resulting cdna molecules are then engineered so that they have RE sites at each end of every molecule, which allows them to be digested and inserted into a vector.

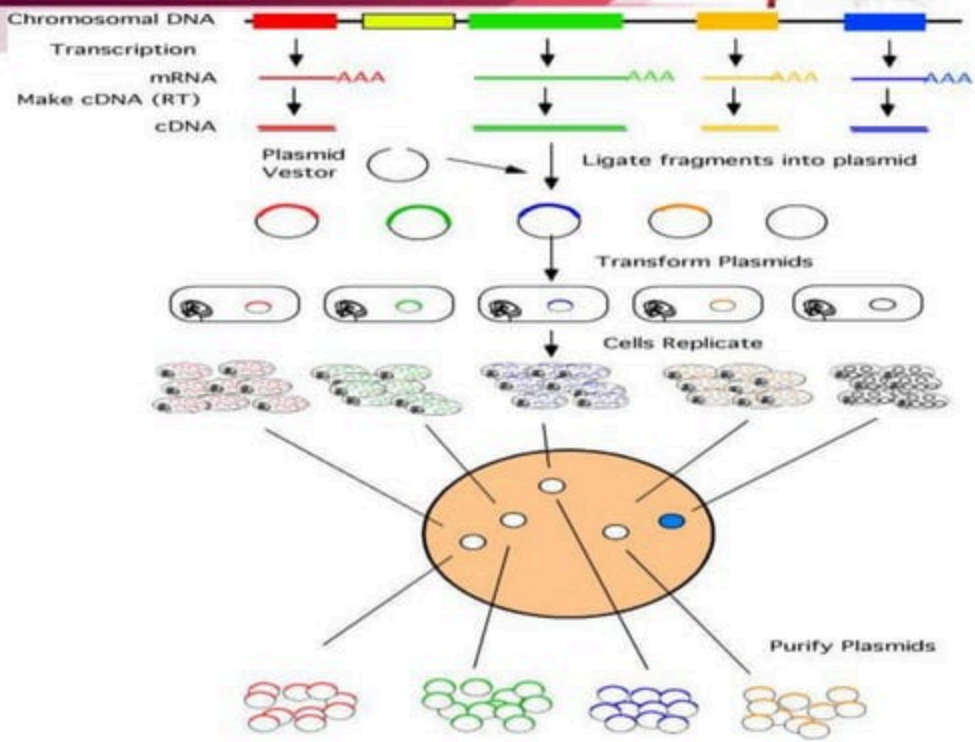
Steps to prepare cdna



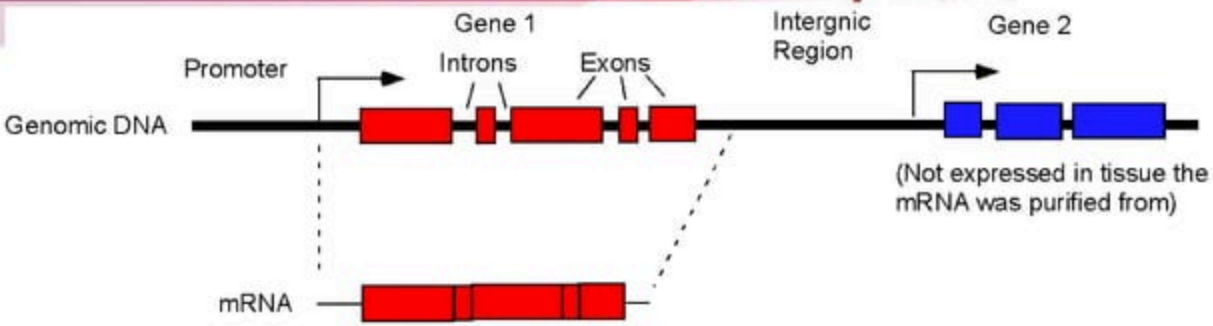
Synthesis of cDNA from mRNA



Construction of cdna library



Differences between a genomic and cDNA library

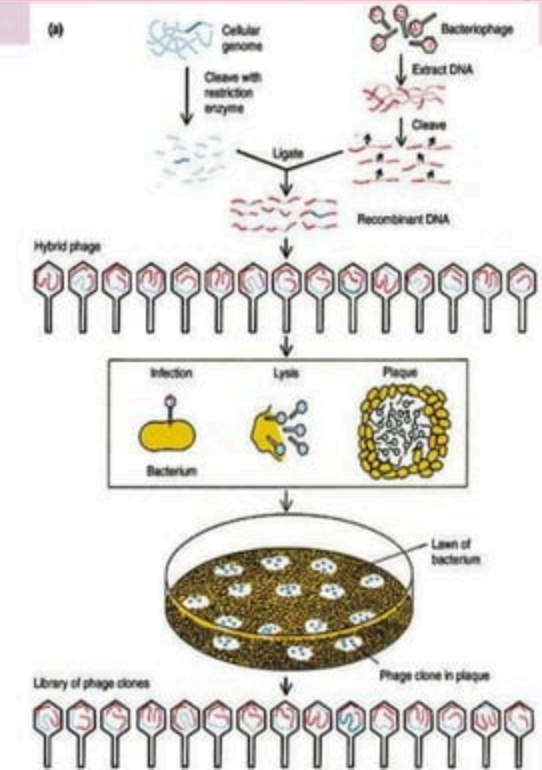


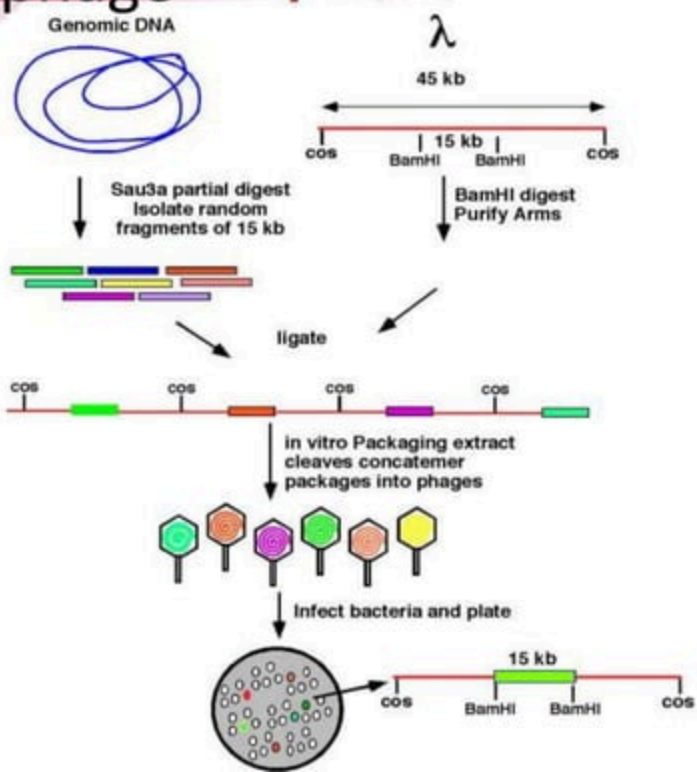
Genomic Library	cDNA Library
Promoters	Expressed genes
Introns	Transcription start sites
Intergenic	Open reading frames (ORFs)
Non-expressed genes	Splice points

TABLE 6.5 The different cloning vectors with the corresponding hosts and the sizes of foreign insert DNAs

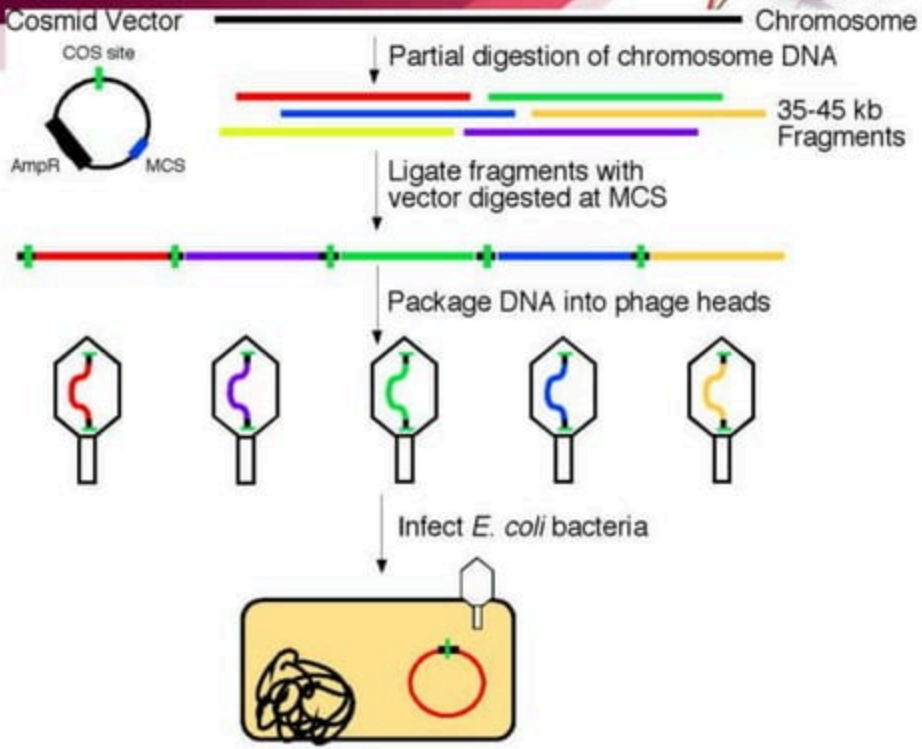
Vector	*Host*	*Foreign insert DNA size*
Phage λ	*E. coli*	5–25 kb
Cosmid λ	*E. coli*	35–45 kb
Plasmid artifical chromosome (PAC)	*E. coli*	100–300 kb
Bacterial artificial chromosome (BAC)	*E. coli*	100–300 kb
Yeast chromosome	*S. cerevisiae*	200–2000 kb

Constructing a genomic library in λ phage

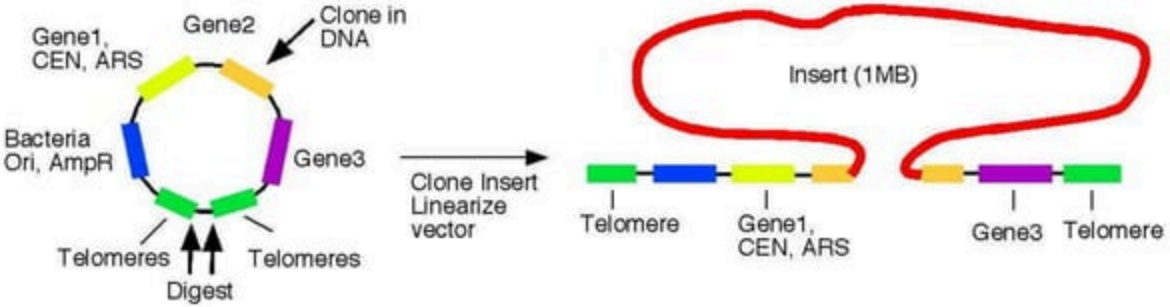




Constructing a genomic library in cosmids



Constructing a genomic library in YACs



Screening a clones

- Expression screening or Direct selection of recombinants
- Insertional selection inactivation method
- Blue white screening
- Colony hybridization
- PCR screening of gene libraries
- Hybrid select/arrest translation
- Screening expression cDNA libraries.

Expression screening

Identify the protein product of an interested gene

1. **Protein activity**
2. **Western blotting using a specific antibody**

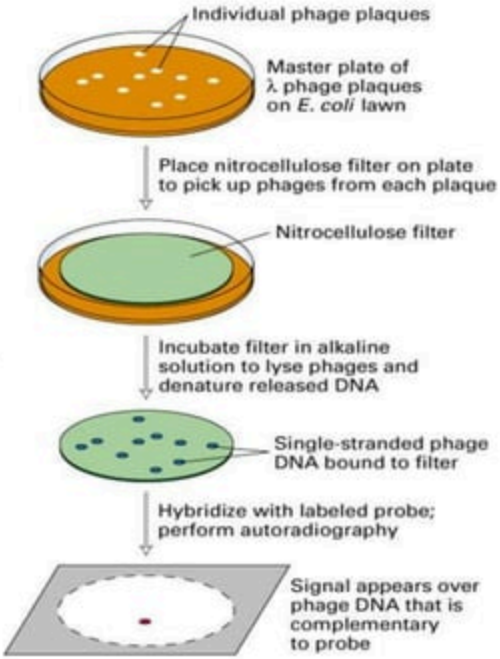
Eg:

- Finding the gene for alanine production
- Grow in alanine deficit medium
- Then they are labelled in the petri plate indicates that gene for alanine production is stored in bacteria.

Colony hybridization

Screening a genomic library using DNA hybridization to a (radio-)labeled DNA probe

Note: a cDNA is commonly (radio-)labeled and used as a DNA probe to screen a genomic library



Hybrid arrest/release

✓Individual Cdna clones or pools of clones can be used to hybridize to mrna preparation

✓**Hybrid arrest**: translate the Mrna population directly, and the inhibition of translation of some products detected.

✓**Hybrid release translation**: purify the hybrids and the hybridized mrnaa released from them and translated, it identifies the protein encoded by the cdna clone.

Uses of gene library

- To obtain the sequences of genes for analysis, amplification, cloning, and expression.
- Once the sequence is known probes, primers, *etc.* can be synthesized for further diagnostic work using, for example, hybridization reactions, blots and PCR.
- Knowledge of a gene sequence also offers the possibility of gene therapy.
- Also, gene expression can be used to synthesize a product in particular host cells, *e.g.* synthesis of human gene products in prokaryotic cells.


THANK
YOU